Incorporation of zidovudine into leukocyte DNA from HIV-1-positive adults and pregnant women, and cord blood from infants exposed *in utero*

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Objective: The nucleoside analog 3′-azido-3′-deoxythymidine (ZDV) has widespread clinical use but also is carcinogenic in newborn mice exposed to the drug *in utero* and becomes incorporated into newborn mouse DNA. This pilot study was designed to determine ZDV incorporation into human blood cell DNA from adults and newborn infants.

Design: In this prospective cohort study, peripheral blood mononuclear cells (PBMC) were obtained from 28 non-pregnant adults and 12 pregnant women given ZDV therapy, six non-pregnant adults with no exposure to ZDV, and six non-pregnant adults who last received ZDV ≥ 6 months previously. In addition, cord blood leukocytes were obtained from 22 infants of HIV-1-positive, ZDV-exposed women and from 12 infants unexposed to ZDV. There were 11 mother–infant pairs involving HIV-1-positive women.

Methods: DNA was extracted from PBMC obtained from non-pregnant HIV-1-positive adults taking ZDV, pregnant HIV-1-positive women given ZDV during pregnancy, and from adults not taking ZDV. Cord blood leukocytes were examined from infants exposed to ZDV *in utero* and from unexposed controls. DNA samples were assayed for ZDV incorporation by anti-ZDV radioimmunoassay (RIA).

Results: The majority (76%) of samples from ZDV-exposed individuals, pregnant women (8 of 12), non-pregnant adults (24 of 28), or infants at delivery (15 of 22), had detectable ZDV–DNA levels. The range of positive values for ZDV-treated adults and infants was 25–544 and 22–452 molecules ZDV/10^6 nucleotides, respectively. Analysis of 11 mother-infant pairs showed variable ZDV–DNA incorporation in both, with no correlation by pair or by duration of drug treatment during pregnancy. Two of the 24 samples from individuals designated as controls were positive by anti-ZDV RIA. The 20-fold range for ZDV–DNA values in both adults and infants suggested large interindividual differences in ZDV phosphorylation.

Conclusions: Incorporation of ZDV into DNA was detected in most of the samples from ZDV-exposed adults and infants. Therefore, the biologic significance of ZDV–DNA damage and potential subsequent events, such as mutagenicity, should be

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further investigated in large cohorts of HIV-positive individuals. The interindividual variability of ZDV incorporation into DNA in humans is considerable and consistent with reported variability in the formation of the ZDV-trisphosphate.

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Introduction

The drug 3′-azido-3′-deoxythymidine (ZDV), a nucleoside analog currently used in the therapy of AIDS, is carcinogenic both in adult mice and in mice exposed to ZDV while *in utero* [1,2,4–6]. In adult female mice, ZDV given in drinking water for 2 years induced vaginal epithelial papillomas and carcinomas [1,2], while ZDV given by the same route for 28 days induced dose-related ZDV incorporation into DNA (formation of ZDV–DNA), cell proliferation, and preneoplastic distribution of epithelial integrins in the vagina [3]. ZDV was also tumorigenic in 1- and 2-year-old male and female offspring of pregnant CD-1 mice that were given daily oral doses of the drug on days 12–18 of gestation [4,5]. Histopathology performed on the offspring demonstrated the presence of statistically significant increases in lung, liver, skin, and reproductive organ tumors [4–6]. Tumor incidences were higher after ZDV exposure of mice *in utero* (13–65%) [4] compared with that in mice receiving lifetime oral ZDV administration from 6 weeks of age (10-22%) [1,2].

Genotoxicity, in the form of DNA adduct formation or incorporation of abnormal nucleosides into DNA, has been shown to be necessary but not sufficient to initiate the multiple critical mutagenic events leading to carcinogenesis [7–9]. In animal and cell culture models, ZDV is clastogenic [3,10,11]; ZDV-induced skin tumors have H-*ras* activating mutations [6], and ZDV-induced mutagenesis of the *HPRT* locus has been reported [12,13]. Incorporation of ZDV into DNA has been demonstrated in multiple cell culture and animal model systems [4,14,15]. Offspring of pregnant mice given transplacental ZDV exposure at tumorigenic doses and examined at birth were shown to have ZDV incorporated into the DNA of multiple organs, some of which were targets for tumorigenicity [4]. Furthermore, ZDV–DNA was observed in nuclear and mitochondrial DNA of brain, lung, liver, kidney, heart, and placenta from fetuses of *Erythrocebus patas* monkeys given approximately 20% of the human daily dose for the last half of gestation [4]. It was not possible to examine tumorigenicity in the monkey. However, the levels of ZDV–DNA in tissues from fetuses of monkey dams given daily ZDV doses that were 80% lower than the human daily dose were higher than the levels found in fetal mouse pups given a dosing regimen that produced tumors.

In the United States, ZDV is used widely for the therapy of HIV-1 infection, although monotherapy is rarely used except to prevent perinatal transmission. Current treatment guidelines from the Centers for Disease Control and Prevention comprise triple drug combination therapy, which includes two nucleoside reverse transcriptase inhibitors and one protease inhibitor. When the nucleoside analog is ZDV, the drug is given in doses of 500–600 mg/day to adults. The Public Health Service recommends treating all HIV-1-positive pregnant women with ZDV during pregnancy and intrapartum and giving the newborn ZDV during the first 6 weeks of life. Administration of ZDV during weeks 14–38 of pregnancy reduces maternal–fetal virus transmission approximately threefold [16,17]. In HIV-1-positive pregnant women, ZDV is the only drug approved for inhibition of viral transmission.

There have been no previous reports demonstrating incorporation of ZDV into human DNA, either in adult HIV-1-positive patients or in children exposed to the drug *in utero*. Awareness of this possibility might be useful for the toxicologist considering metabolism and estimating mutagenic potential. Consequently, this study was designed to measure ZDV incorporation into DNA in leukocytes of ZDV-exposed humans and to determine the frequency of its incorporation in newborns exposed to ZDV *in utero*. We show here that ZDV is incorporated into leukocyte DNA of most individuals receiving ZDV therapy, including infants exposed to the drug *in utero*. Similar or lower levels of ZDV–DNA have been observed in mice given carcinogenic and mutagenic doses of ZDV, suggesting that further study of the biological consequences of ZDV-induced DNA damage in the human population is warranted.

Methods

Blood samples: source and processing

Peripheral blood samples from adult patients with AIDS (n = 56), including 10 females and 46 males, were obtained from Rush-Presbyterian–St Luke's Medical Center (Chicago, Illinois). Patients were taking ZDV with or without other antiretroviral drugs in combination. The demographic background included 33 Caucasians, 14 African-Americans, 8 Hispanics, and 1 Asian. Most of the patients received a standard dose of 600 mg ZDV/day (200 mg three times daily or 300 mg two times daily) but two patients were currently taking 300 mg ZDV/day and one patient was taking 400 mg ZDV/day. Sufficient DNA for assay was obtained from 40 of the 56 patients. For these 40 individuals, 28 were receiving ZDV therapy at the time of sampling (including the three on the reduced doses), six never received ZDV, and six received their last dose of ZDV 6 months previously.

Peripheral blood and cord blood samples were obtained from 11 mother–infant pairs, one additional mother, and 11 additional infants of HIV-1-positive women who delivered at the Los Angeles and University of Southern California (LAC+USC) Medical Center and were enrolled in an ongoing perinatal transmission study at the Comprehensive Maternal–Child HIV management and Research Center, University of Southern California, (Los Angeles, California). Whole blood was collected in heparinized tubes and shipped overnight to the central processing laboratory. Pregnant HIV-1-positive women were prescribed ZDV as recommended by the US Public Health Service: 600 mg ZDV given in three doses daily for the last 14–38 weeks of pregnancy, depending on when the patient was identified or referred for HIV-1 care, and intravenous infusion of 1 mg ZDV/h per kg body weight (approximately three times higher than the daily dose) during labor and delivery. The natural history of the disease was followed in these patients and their children. As control for the ZDV-exposed infants, 12 cord blood samples were obtained from normal pregnancies from presumed HIV-1-negative women with no ZDV exposure at Fairfax Inova Hospital, Department of Neonatology, Falls Church, Virginia.

Peripheral blood mononuclear cells (PBMC), prepared from all adult blood samples, and cord blood leukocytes were separated from whole blood on lymphocyte preparation medium (Lymphoprep, Organon Teknika, Rockville, MD) and cryopreserved in freezing medium (RPMI) containing 20% fetal calf serum and 10% dimethylsulfoxide (Sigma, St Louis, MO).

All pregnant and non-pregnant adult patients participating in these studies gave informed consent. Blood samples were collected with internal review board approval at the different institutions, which included the Human Investigative Committee at Rush-Presbyterian–St Luke's Medical Center and the Internal Review Boards at USC School of Medicine and Fairfax Inova Hospital. Additional consent for the NCI participation was given by the Office of Human Subjects Research Internal Review Board.

Preparation of DNA

DNA was isolated by CsCl gradient centrifugation [18] of nuclei prepared from PBMC or cord blood lymphocytes by lysing (330 mM sucrose, 5% triton X-100, 0.3 mM $CaCl_2$, 1 mM potassium phosphate buffer, pH 7.4) and washing (330 mM sucrose, 0.3 mM $CaCl_2$, 1 mM potassium phosphate buffer, pH 7.4). Fractionation of gradients yielded a band of DNA that was dialyzed against water. The concentration of DNA was determined by its absorbance at 260 nm (A_{260}) and adjusted with deionized water to 30 μg DNA/ml.

Incorporation of ZDV into DNA

DNA samples, in distilled water, were sonicated for 30 s and boiled for 5 min. Portions of DNA were subsequently assayed by competitive anti-ZDV radioimmunoassay (RIA) as previously described [4] using a polyclonal anti-ZDV antibody (Sigma). Briefly, the anti-ZDV antibody, reconstituted and diluted 1 : 7500 in 30 ml 10 mM TRIS buffer pH 8.0, was incubated with either standard ZDV plus 3 μg sonicated and boiled calf thymus carrier DNA (Sigma) or 3 μg sample DNA from ZDV-exposed or unexposed patients for 90 min at 37°C in 10mM TRIS buffer pH 8.0. Tracer was added as approximately 20 000 c.p.m. [^{3}H]-ZDV (20 Ci/mmol; Moravek Biochemicals, Mountain View, CA) in 100 μl to each tube together with 100 μl of the secondary antibody, goat antirabbit immunoglobulin G (Sigma) reconstituted in 12 ml 10mM TRIS buffer, pH 8.0. The mixture was incubated for 25 min at 4°C and tubes were centrifuged at $2000 \times g$ for 15 min at 4°C. The resulting supernatant was decanted, the pellets were dissolved in 100 mM NaOH, and the radioactivity was counted in a liquid scintillation counter. The concentration of standard ZDV required to inhibit antibody binding by 50% was 490.0 ± 183.3 fmol ZDV [mean ± SD (standard deviation)] (n = 20). The lower limit of detection was 15.2 ± 8.4 molecules ZDV/10^6 nucleotides (n = 20). Unless otherwise indicated, each sample was assayed in three separate radioimmunoassays and the mean and SE calculated.

Statistical methods

To assess the dose–response relationship between ZDV incorporation into DNA and time on ZDV treatment, the Jonckheere–Terpstra non-parametric trend test [19] was employed for analysis of categorized data and the Pearson correlation coefficient and Spearman rank correlation coefficient [19] were employed for analysis of individual measurements. Two-sided *P* values are reported for all analyses.

Results

Incorporation of ZDV in adult patients

Sufficient DNA to perform ZDV–DNA determinations by RIA was available from 40 adult patient samples. Samples from patients who had never received ZDV (n = 6) and patients who had a lapse of ≥ 6 months between the time of last ZDV treatment and sampling (n = 6) were considered to be controls. Of the six samples from patients who had never received ZDV, five were negative by ZDV–DNA RIA and one gave a positive value of 45.6 ± 15.2 molecules ZDV/10^6 nucleotides. For the six patients that had previously taken ZDV, time since last treatment was 6 and 17 months, and 2, 3, 5, and 10 years prior to sampling, respectively. Five of the samples from these patients were negative, but the PBMC sample from the patient who received ZDV 2 years prior to sampling gave a value of 296.3 ± 36.5 molecules ZDV/10^6 nucleotides.

Blood samples obtained from 28 patients who were receiving ZDV at the time their blood was drawn yielded sufficient DNA to assay (Table 1). The length of ZDV treatment ranged from 1 to 96 months. In 24 (86%) of these samples, measurable values by ZDV–DNA RIA were obtained, and in four samples, from patients receiving ZDV from 9 to 48 months, levels were not measurable (Table 1). The increase in mean ZDV–DNA levels with increasing months of ZDV therapy, using the categorized data presented in Table 1, was not significant by non-parametric trend test ($P = 0.18$ when all ZDV–DNA values were included in the analysis and $P = 0.10$ when the analysis excluded ZDV–DNA values less than 30 molecules ZDV/10^6 nucleotides). By combining the ZDV–DNA values for all ZDV-treated patients (n = 28), and assigning the four non-detectables a value halfway between zero and the detection limit (see Table 1), a value of 145.6 ± 27.8 molecules ZDV/10^6 nucleotides was obtained. Of the 28 patients taking ZDV at the time blood was drawn, the daily dose of ZDV was 600, 300, and 400 mg in 25, two, and one patients, respectively. Therefore, the difference in dose level was only twofold, while the interindividual variability in ZDV–DNA level was over 20-fold (Table 1). Although low ZDV–DNA values were not necessarily seen only among the individuals taking the drug for the shortest time (Fig. 1), a weak correlation was observed between duration of ZDV dosing and ZDV–DNA levels when analyzed by Pearson's correlation coefficient with values below 30 molecules ZDV/10^6 nucleotides eliminated (see legend to Fig. 1).

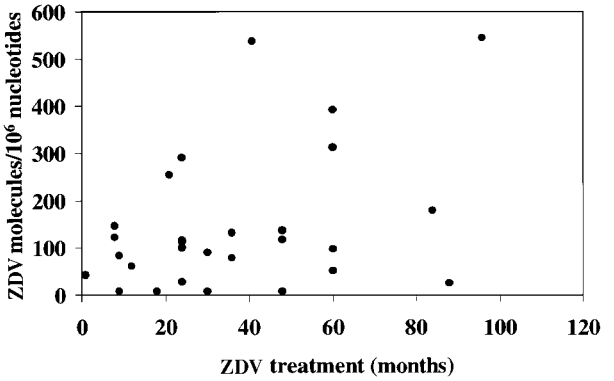

Fig. 1. Incorporation of zidovudine (ZDV) into the DNA of peripheral blood mononuclear cells from 28 HIV-1-positive adults, 25 of whom were receiving 600 mg ZDV daily. If values below 30 molecules ZDV/10^6 nucleotides were not included in the calculations, very weak correlations are observed between duration of ZDV treatment and ZDV incorporation into DNA by Pearson's correlation coefficient (0.50; $P = 0.019$) and Spearman rank correlation (0.39; $P = 0.069$).

Incorporation of ZDV in HIV-1-positive mothers and in infants

Peripheral blood was obtained at delivery from 12 HIV-1-positive women given a dose of 600 mg ZDV daily from 3 weeks to 9 months of pregnancy. Data for administration of ZDV prior to pregnancy were not

Table 1. Incorporation of zidovudine (ZDV) into peripheral blood mononuclear cell DNA from 28 adult HIV-1-positive patients grouped by duration of therapy.

	Incorporation[a] into DNA (molecules ZDV/10^6 nucleotides) after therapy (months)			
	1–12	13–24	25–48	49–96
	43.0 ± 21.7	27.7 ± 11.5	77.8[b]	24.6 ± 10.3
	60.4 ± 11.3	100.0 ± 9.2	89.5 ± 24.8[c]	50.4 ± 24.8
	83.0 ± 16.7	112.6 ± 17.2	116.7 ± 37.6	97.4 ± 56.3
	121.5 ± 19.1	115.9 ± 17.2	132.4 ± 50.4	178.5 ± 58.3
	147.0 ± 37.8	253.7 ± 27.3	137.1 ± 24.0	313.0 ± 51.0
	ND	290.7 ± 168.1	537.0 ± 145.0	391.7 ± 183.9
	–	ND	ND	544.4 ± 71.8
	–	–	ND	–
Overall mean	77.1 ± 20.9	129.7 ± 40.3	138.2 ± 59.7	228.5 ± 73.6
Median	71.7	112.6	103.1	178.5

[a]Incorporation as mean ± SE of three assays unless indicated otherwise. For the calculation of means for each group, the ND samples were given a value of 7.6 molecules ZDV/10^6 nucleotides, the value halfway between zero and the lower limit of detection. [b]Assayed once. [c]Assayed twice.

Table 2. Incorporation of zidovudine (ZDV) into peripheral blood mononuclear cell DNA from 12 HIV-1-positive pregnant women grouped by duration of ZDV therapy administered while pregnant.

	Incorporation[a] into DNA (molecules ZDV/10^6 nucleotides) after therapy (months) prior to delivery		
	<1–3	4–5.5	6–9
	35.5 ± 27.8	79.6 ± 14.7	100.4 ± 51.3
	92.6 ± 23.0	ND	105.2 ± 52.9
	109.0 ± 41.7	ND	214.8 ± 48.6
	183.4 ± 13.6	ND	–
	ND	–	–
Overall mean	85.6 ± 30.6	25.6 ± 18.0	140.1 ± 37.4
Median	92.6	7.6	105.2

[a]Incorporation as mean ± SE of three assays. For the calculation of means for each group, the ND samples were given a value of 7.6 molecules ZDV/10^6 nucleotides, the value halfway between zero and the lower limit of detection.

considered here, and information concerning compliance was not available. In addition, the amount of ZDV received during labor varied with the time of labor and delivery. Four individuals (33%) did not exhibit measurable ZDV incorporation in DNA extracted from the PBMC, while the remaining eight individuals had ZDV–DNA values in PBMC ranging from 35.5 to 214.9 molecules ZDV/10^6 nucleotides (Table 2). The mean ZDV–DNA value for all 12 ZDV-treated women was 79.3 ± 20.3 molecules ZDV/10^6 nucleotides.

Cord blood was obtained at delivery from 12 ZDV-unexposed infants (11 of HIV-1-negative mothers and one of an HIV-1-positive mother). All 12 of these control samples were negative by ZDV–DNA RIA. Cord blood was also obtained at delivery from 22 infants of HIV-1-positive women who received 600 mg ZDV daily from the time of identification to delivery (Table 3). Fifteen of these samples (68%) were positive by ZDV–DNA RIA. There was no correlation between ZDV–DNA levels and the duration of ZDV administration during pregnancy (data not shown). In analyses of the duration of *in utero* exposure and ZDV–DNA levels, the non-parametric trend test gave a P value of 0.23, the Pearson correlation coefficient was –0.37 ($P = 0.089$) and the Spearman rank correlation coefficient was –0.27 ($P = 0.22$). The mean value for the 22 *in utero* exposures was 83.8 ± 26.1 molecules ZDV/10^6 nucleotides compared with 145.6 ± 27.8 molecules ZDV/10^6 nucleotides observed for the 28 adults (P= 0.1 by Student's t-test).

This study also allowed us to compare ZDV–DNA levels in maternal peripheral blood and infant cord blood for each of 11 mother–infant pairs (Fig. 2). For one pair, both mother and baby were negative, and for five pairs both had measurable values. For three pairs, the infant was positive and the mother was negative and for two pairs the mother was positive while the

Table 3. Incorporation of zidovudine (ZDV) into leukocyte DNA from cord blood of 22 newborns from HIV-positive mothers grouped by duration of ZDV therapy administered to their mothers before delivery.

	Incorporation[a] into DNA (molecules ZDV/10^6 nucleotides) after therapy (months) prior to delivery		
	< 1–3	4–5.5	6–9
	21.8 ± 12.5	35.0 ± 16.8	23.6 ± 18.6
	63.1 ± 33.4	45.7 ± 33.5	28.7 ± 18.6
	64.2 ± 19.3	56.5 ± 23.9	68.9 ± 27.2
	68.0 ± 16.0	95.2 ± 14.0	ND
	313.3 ± 121.8	111.1 ± 22.3[b]	ND
	344.5 ± 133.3	451.8 ± 83.0	–
	ND	ND	–
	–	ND	–
	–	ND	–
	–	ND	–
Overall mean	126.1 ± 53.2	82.6 ± 42.7	27.3 ± 11.2
Median	64.2	40.3	23.6

[a]Incorporation as mean ± SE of three assays unless indicated otherwise. For the calculation of means for each group, the ND samples were given a value of 7.6 molecules ZDV/10^6 nucleotides, the value halfway between zero and the lower limit of detection. [b]Assayed twice.

infant was negative. Therefore, there was no consistency in ZDV–DNA levels by mother–infant pair. In addition, there was no correlation between ZDV–DNA level and duration of ZDV treatment. Samples were positive in 64% of the mothers and 73% of the newborns, and the mean value for the 11 infants was 86.4 ± 37.3 molecules ZDV/10^6 nucleotides, essentially the same as the 64.2 ± 18.5 molecules ZDV/10^6 nucleotides for their mothers ($P > 0.05$). The data demonstrate appreciable interindividual variability for ZDV incorporation into DNA in both mothers and infants.

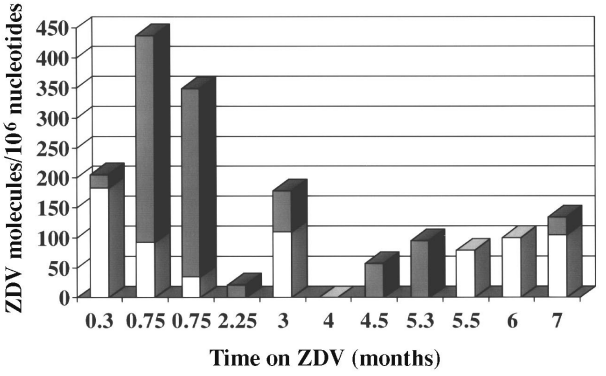

Fig. 2. Incorporation of zidovudine (ZDV) into the DNA of peripheral blood mononuclear cells in HIV-1-positive mothers (□) and cord blood of their infants (■) taken at delivery. ZDV was given continuously up to delivery for the intervals shown on the abscissa. ZDV–DNA was not detectable in either the mother or the infant for the pregnant woman who received ZDV for 4 months. Values are the means of three assays; assay variability is shown in Table 2.

Discussion

This study demonstrates that ZDV is incorporated into peripheral blood DNA of adult males and pregnant and non-pregnant adult females receiving ZDV as treatment for HIV-1 infection or to prevent maternal–fetal virus transmission. Incorporation of ZDV into DNA was also shown in cord blood leukocytes from infants of ZDV treated mothers. Most DNA samples from ZDV-treated individuals (86% from non-pregnant adults, 66% from pregnant women, and 68% from infants) had measurable ZDV–DNA levels by ZDV-RIA. Although this is a cross-sectional study with evaluations performed only at a single time and with no long-term follow-up, there may be significant implications for infants exposed *in utero* to ZDV and other nucleoside analogs.

Since ZDV is a chain terminator and the RIA for ZDV uses DNA that is denatured and sonicated, it is likely that the antiserum is recognizing terminal ZDV on oligonucleotides. It is possible that the antiserum might also recognize ZDV attached to proteins, and it is certain that free ZDV will be recognized. Therefore, to ensure the integrity of these results, stringent methods of DNA purification have been applied. Once the ZDV trisphosphate is incorporated as if it were a normal base, DNA repair mechanisms are likely to be induced. Currently, little is known regarding intracellular mechanisms for removal of ZDV from primate DNA, but these might include recombinational repair and gap-filling measures.

Based on previous experiments in animal models, the incorporation of ZDV into human DNA might allow subsequent occurrence of mutagenic events known to be associated with the process of tumor development. Tumorigenicity and mutagenicity have been demonstrated in ZDV-exposed mice in which ZDV was administered both to adult animals [1,2] and transplacentally to the fetus [4]. Incorporation of ZDV into DNA has also been demonstrated in transplacentally exposed mouse and monkey fetuses [4]. Genotoxicity, in the form of DNA adduct formation and drug incorporation into DNA, has been demonstrated extensively in the carcinogenesis literature to comprise a necessary but not sufficient prelude to tumor formation in many species of animals [7–9]. In animal models, compounds that inhibit DNA damage also inhibit tumor formation [9]; in humans, inability to repair DNA damage in skin results in accelerated latency and increased yield of skin cancers [20]. Therefore, ZDV incorporation into DNA in humans may be an indicator of potential cancer risk.

In addition to suggesting possible tumorigenic potential for ZDV in human patients, the data presented here provide some insights into interindividual variability in ZDV metabolism. Although the daily ZDV dose received by 92% of the 40 pregnant and non-pregnant adult patients was 600 mg, there was a large range in the amount of ZDV incorporation into DNA. It is possible that some of this variability is related to non-compliance with the prescribed treatment schedule. However, since the lowest positive samples were approximately 25 molecules ZDV/10^6 nucleotides and the highest value was 544.4 molecules ZDV/10^6 nucleotides, it seems unlikely that occasional lapses in compliance would produce such large differences. The specific drug pharmacokinetics of pregnancy, the extent of ZDV infusion during labor and delivery, the duration of ZDV therapy prior to pregnancy, and fetal ZDV metabolism may all contribute to the range of ZDV–DNA levels observed in cord blood leukocytes. In addition, this study was limited to evaluations of leukocytes and, therefore, data concerning the extent of possible ZDV incorporation into DNA in non-hematologic human tissues are not available.

Variability in ZDV metabolism has been reported previously. For a single dose of ZDV, variations of more than 10-fold have been observed in mean daily ZDV plasma concentrations [21], and plasma pharmacokinetics are not necessarily correlated with clinical effect [22]. Approximately 13-20% of a single ZDV dose is excreted in the urine unchanged, while 60-75% is excreted as the ZDV glucuronide derivative (ZDVG) and about 2% as the 3′-amino-3′-deoxythymidine (AMT) [21,23]. Hepatic AMT metabolite formation is considered to involve multiple cytochrome P450 enzymes [24]. Variability in these major metabolic pathways will determine how much ZDV is available to become incorporated into DNA.

Incorporation of ZDV into DNA requires intracellular mono-, bis- and trisphosphorylation performed by three different kinases [24]. In one study, a 1000-fold increase in extracellular ZDV increased the intracellular ZDV monophosphate concentration by 150-fold [24]; this and other evidence suggest that the extent of phosphorylation is variable and may be regulated more by kinase activity and less by drug dose [24]. The ZDV monophosphate accumulates inside the cell and accounts for approximately 95% of the total ZDV phosphate derivatives, while the bis- and trisphosphates are present in equal proportions [24]. Improvement in asymptomatic HIV-1-positive patients, indicated by CD4/CD8 cell ratio, was shown to be associated with increased levels of excreted phosphorylated ZDV metabolites [22]. Conditions of general health (cirrhosis of the liver, kidney failure, HIV-1 status) can alter the extent of phosphorylation [21], and it appears that intracellular phosphorylation is more efficient in HIV-1-infected individuals with CD4 counts $< 200 \times 10^6$ cells/l than in healthy volunteers [21,23,24]. In one study [21], intracellular levels of ZDV trisphosphate, the direct precursor for ZDV incorporation into DNA, ranged from 3 to 326 fmolecules/10^6 cells for

12 HIV-1-positive volunteers. The magnitude of this difference reflects the range of ZDV–DNA values reported here and suggests that the interindividual variability in ZDV incorporation into DNA may have a metabolic basis.

Overall, these data raise the possibility that the presence of extensive ZDV incorporation into human DNA may be cumulative, with potential long-term consequences such as mutagenicity and tumorigenicity. To-date, there are published epidemiologic studies suggesting that ZDV treatment of adults with HIV-1 is not associated with either an increased risk of developing lymphoma [25,26] or a decrease in the latency of Kaposi's sarcoma [27]. However, increases in AIDS-related malignancies, presumably as a result of immunosuppression, are well documented. Of the groups investigated here, the HIV-1-negative children of HIV-1-positive mothers are perhaps the most likely to sustain potential mutagenic and carcinogenic effects since they are likely to have a normal lifespan, with over 30 years available for cancers to develop. Recently published data from the Pediatric AIDS Clinical Trial Group [28] indicated no adverse outcomes in infants exposed *in utero* to ZDV, but follow-up at present is still not more than 5 years for most children. Whereas the impressive benefits of ZDV given to pregnant women to reduce fetal HIV-1 transmission far outweigh the known risks, ZDV-exposed children should be followed for potentially harmful long-term effects that may result from ZDV treatment during pregnancy.

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